CLAIMS

1. A method for detecting and determining species source of eubacterial DNA in a sample, comprising:

amplifying template DNA in a sample using a real-time polymerase chain reaction (PCR), wherein the PCR employs **primers** and at least two **fluorogenic probes**, wherein the **primers** if in the presence of a *S. aureus* 16S rRNA gene amplify a segment of the gene comprising a conserved region and a first divergent region, wherein the conserved region comprises at least 18 contiguous nucleotides which are at least 80% identical among at least 10 eubacterial species wherein the first divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region found in *Bradyrhizobium japonicum* 16S rRNA gene; wherein each of the **fluorogenic probes** comprises a reporter dye and a quencher dye, a first of the two fluorogenic probes hybridizing to the conserved region and the second of the two fluorogenic probes hybridizing to a third divergent region of a first species of eubacteria, wherein the reporter dyes of the first and the second probes have non-overlapping emission spectra;

monitoring fluorescence emissions of the reporter dyes;

determining presence of eubacteria in the sample if emissions characteristic of the reporter dye of the first probe are detected; and

determining presence of the first species of eubacteria in the sample if emissions characteristic of the reporter dye on the second probe are detected.

2. The method of claim 1 wherein a third fluorogenic probe is employed in the real-time PCR, wherein the third fluorogenic probe hybridizes to a fourth divergent region of 16S rRNA gene in a second species of eubacteria and wherein the reporter dye of the third fluorogenic probe has a non-overlapping emission spectrum from the reporter dyes of the first and second probes.
3. The method of claim 2 wherein a fourth fluorogenic probe is employed in the real-time PCR, wherein the fourth fluorogenic probe hybridizes to a fifth divergent region of 16S rRNA gene in a third species of eubacteria and wherein the reporter dye of the

fourth fluorogenic probe has a non-overlapping emission spectrum from the reporter dyes of the first, second, and third probes.

4. The method of claim 1 wherein the segment of *S. aureus* 16S rRNA gene comprises nucleotides 890 to 1051.
5. The method of claim 1 wherein the conserved region of *S. aureus* 16S rRNA gene comprises nucleotides 1002 to 1024.
6. The method of claim 1 wherein the first divergent region comprises nucleotides 945 to 978 of *S. aureus* 16S rRNA gene.
7. The method of claim 1 wherein the sample is a treated blood sample.
8. The method of claim 7 wherein the blood sample is from a patient suspected of systemic bacteremia.
9. The method of claim 7 wherein the sample was treated to extract DNA from cells.
10. The method of claim 1 wherein the sample is urine.
11. The method of claim 1 wherein the sample is cerebrospinal fluid.
12. The method of claim 1 wherein the primers comprise primers p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)
13. The method of claim 1 wherein the segment amplified is at least 125 bp.
14. The method of claim 1 wherein the segment amplified is at least 150 bp.
15. The method of claim 1 wherein the segment amplified is at least 160 bp.
16. A method for detecting and determining species source of eubacterial DNA in a sample, comprising:

filtering a real-time PCR reaction mixture to remove double stranded DNA contaminants having a length of $\geq$ 125 bp to form a filtrate, wherein the PCR reaction mixture comprises primers and at least two fluorogenic probes, wherein the primers if in the presence of a *S. aureus* 16S rRNA gene amplify a segment of the gene comprising a conserved region and a first divergent region, wherein the conserved region comprises at least 18 contiguous nucleotides which are at least 80% identical among at least 10 eubacterial species, wherein the first divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region of *Bradyrhizobium japonicum* 16S rRNA gene, wherein each of the probes comprises a reporter dye and a quencher dye, a

first of the two fluorogenic probes hybridizing to the conserved region and the second of the two fluorogenic probes hybridizing to a third divergent region of a first eubacterial species, wherein the reporter dyes of the first and the second probes have non-overlapping emission spectra;

adding a sample comprising template DNA to the filtrate;

amplifying template DNA in the filtrate;

monitoring fluorescence emissions of the reporter dyes;

determining presence of eubacteria in the sample if emissions characteristic of the reporter dye of the first probe are detected; and

determining presence of the first species of eubacteria in the sample if emissions characteristic of the reporter dye on the second probe are detected.

17. The method of claim 16 wherein a third fluorogenic probe is present in the real-time PCR reaction mixture, wherein the third fluorogenic probe hybridizes to a fourth divergent region of a 16S rRNA gene in a second species of eubacteria and wherein the reporter dye of the third fluorogenic probe has a non-overlapping emission spectrum from the first and second probes.
18. The method of claim 17 wherein a fourth fluorogenic probe is present in the real-time PCR reaction mixture, wherein the fourth fluorogenic probe hybridizes to a fifth divergent region of a 16S rRNA gene in a third species of eubacteria and wherein the reporter dye of the fourth fluorogenic probe has a non-overlapping emission spectrum from the first, second, and third probes.
19. The method of claim 16 wherein the segment comprises nucleotides 890 to 1051 of *S. aureus* 16S rRNA gene.
20. The method of claim 16 wherein the conserved region comprises nucleotides 1002 to 1024 of *S. aureus* 16S rRNA gene.
21. The method of claim 16 wherein the first divergent region comprises nucleotides 945 to 978 of *S. aureus* 16S rRNA gene.
22. The method of claim 16 wherein the sample is a treated blood sample.
23. The method of claim 22 wherein the blood sample is from a patient suspected of systemic bacteremia.

24. The method of claim 22 wherein the sample was treated to extract DNA therefrom.
25. The method of claim 16 wherein the sample is urine.
26. The method of claim 16 wherein the sample is cerebrospinal fluid.
27. The method of claim 16 wherein the primers comprise primers p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)
28. The method of claim 16 wherein the segment amplified is at least 125 bp.
29. The method of claim 16 wherein the segment amplified is at least 150 bp.
30. The method of claim 16 wherein the segment amplified is at least 160 bp.
31. The method of claim 16 wherein the conserved region is at least 80% identical among at least 14 eubacterial species.
32. The method of claim 1 wherein the conserved region is at least 80% identical among at least 14 eubacterial species.
33. The method of claim 1 wherein the first divergent region comprises at least 15 contiguous nucleotides.
34. The method of claim 1 wherein the first divergent region comprises at least 20 contiguous nucleotides.
35. The method of claim 1 wherein the first divergent region comprises at least 25 contiguous nucleotides.
36. The method of claim 1 wherein the first divergent region comprises at least 30 contiguous nucleotides.
37. The method of claim 16 wherein the first divergent region comprises at least 15 contiguous nucleotides.
38. The method of claim 16 wherein the first divergent region comprises at least 20 contiguous nucleotides.
39. The method of claim 16 wherein the first divergent region comprises at least 25 contiguous nucleotides.
40. The method of claim 16 wherein the first divergent region comprises at least 30 contiguous nucleotides.
41. The method of claim 1 wherein the first divergent region differs by at least 4 nucleotides from the second divergent region.

42. The method of claim 16 wherein the first divergent region differs by at least 4 nucleotides from the second divergent region.

43. A pair of polymerase chain reaction primers for amplifying a segment of a 16S rRNA gene of eubacteria comprising a conserved region and a divergent region, said pair comprising primers p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)

44. A kit for detecting bacteremia, comprising:

a pair of primers that amplify a segment of a *S. aureus* 16S rRNA gene if in the presence the gene, said segment comprising a conserved region and a first divergent region, wherein the conserved region is common to at least 10 species of eubacteria, wherein the first divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from a second divergent region of 16S rRNA gene from *Bradyrhizobium japonicum;* and

at least two fluorogenic probes, each probe comprising a reporter dye and a quencher dye, a first of the two fluorogenic probes hybridizing to the conserved region and a second of the two fluorogenic probes hybridizing to a third divergent region in a first species of eubacteria, wherein said third divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region, wherein the reporter dyes of the first and the second probes have non-overlapping emission spectra.

45. The kit of claim 44 further comprising:

a third fluorogenic probe, wherein said third fluorogenic probe hybridizes to a fourth divergent region of 16S rRNA in a second species of eubacteria, wherein said fourth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region;

and wherein the reporter dye of the third fluorogenic probe has a non-overlapping emission spectrum from the dyes on the first and second probes.

46. The kit of claim 44 further comprising:

a fourth fluorogenic probe, wherein said fourth fluorogenic probe hybridizes to a fifth divergent region of 16S rRNA in a third species of eubacteria, wherein said fifth divergent region comprises at least 10 contiguous nucleotides and differs by at least 3 nucleotides from the second divergent region, and wherein the reporter dye of the fourth fluorogenic probe has a non-overlapping emission spectrum from the dyes of the first, second, and third probes.

47. The kit of claim 44 wherein the pair of primers are p890F and p1033R (SEQ ID NO: 1 and 2, respectively.)
48. The kit of claim 44 wherein the conserved region comprises nucleotides 1002 to 1024 of *S. aureus* 16S rRNA gene.
49. The kit of claim 44 wherein the first divergent region comprises nucleotides 945 to 978 of *S. aureus* 16S rRNA gene.
50. The kit of claim 44 further comprising a filter for removing double stranded DNA $\geq$ 125 bp in length.
51. The kit of claim 44 further comprising written instructions for analyzing eubacterial infections using the primers and probes.
52. The kit of claim 44 further comprising reagents for carrying out real-time PCR.